respectfully request a four-month extension of time to extend the statutory period for filing a Brief on Appeal for four months, from April 23, 1995, to August 23, 1995. A Petition for Extension of Time and the appropriate fee are being filed concurrently. Applicants are filing this Response in lieu of the Brief on Appeal.

Applicants also include herewith the required fee under 37 C.F.R. § 1.17(r), as required by 37 C.F.R. § 1.129(a), to have this Response entered and to have the finality of the most recent Office Action automatically withdrawn.

Remarks

Claims 1, 2, 4, 7-14, and 17-24 are pending in the application.

The Amendment After Final Rejection filed February 2, 1995, and received in the U.S. Patent and Trademark Office on February 6, 1995 was entered upon the filing of the Notice of Appeal, as per the Advisory Action mailed from the U.S. Patent and Trademark Office on February 21, 1995.

The issues for reconsideration are whether Claims 1, 2, 4, 7-14, 17-20, 22 and 23 are enabled under 35 U.S.C. 112, first paragraph; whether Claims 1, 2 and 4 are obvious under 35 U.S.C. 103 over King; and whether Claims 1, 2, 4, 7-14 and 17-24 are obvious under 35 U.S.C. 103 over WO 90/11092 in view of Huylebroeck *et al*.

Rejection of Claims 1, 2, 4, 7-14, 17-20, 22 and 23 under 35 U.S.C. 112, first paragraph

The Examiner has stated that Claims 1, 2, 3, 7-14, 17-20, 22 and 23 are rejected in that "the disclosure is enabling only for claims limited to a method of immunizing vertebrates by administering a DNA transcription unit encoding H1 and H7 hemagglutinin antigens" (Advisory Action mailed from the U.S. PTO on February 21, 1995).

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Applicants respectfully disagree with this assessment. Applicants suggest that the particular subtypes of influenza are representative of the types of influenza against which vertebrates can be protected, and that influenza is representative of the pathogens against which vertebrates can be protected.

A. Claims 1, 2, 4, 7-14 and 17-18

1. Subject Matter of Claims and Support in the Specification

Claim 1 pertains to a method of immunizing a vertebrate against an infectious agent, the method comprising administering a DNA transcription unit which comprises DNA encoding an antigen linked to a promoter region, thereby eliciting a humoral immune response and/or a cell-mediated immune response against the desired antigen, whereby the vertebrate is protected from disease caused by the infectious agent. (See the Specification at page 2, lines 5-16; and page 4, lines 5-11).

Claim 11 also pertains to a method of immunizing a vertebrate against an infectious agent, the method comprising administering *to a mucosal surface* a DNA transcription unit which comprises DNA encoding an antigen linked to a promoter region, thereby eliciting a humoral immune response and/or a cell-mediated immune response against the desired antigen, whereby the vertebrate is protected from disease caused by the infectious agent. (See the Specification at page 2, lines 5-21; page 4, lines 5-11; and page 6, lines 4-11).

In one embodiment of the invention, the DNA transcription unit is of nonretroviral origin. (See the Specification at Examples 6 through 8, pages 12-16 and 21-25; Figures 4A, 4B, and 4C; and Figure 5, for Claims 2 and 12.)

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In another embodiment of the invention, the infectious agent is a virus. (See the Specification at page 2, lines 22-24; page 5, lines 19-33; and Examples 1 through 8, pages 7-25, for Claims 4 and 14.)

In another embodiment of the invention, the vertebrate is a mammal. (See the Specification at page 2, lines 15-16; page 5, lines 5-11; and Examples 6 and 7, pages Example 7, pages 12-16 and 21-24, for Claims 7 and 17.) In another embodiment, the mammal is a human. (See the Specification at page 2, lines 15-16; and page 4, lines 5-11, for Claims 8 and 18.)

The DNA transcription unit can be administered through different routes, including intranasal, intravenous, intramuscular, intraperitoneal, intradermal and subcutaneous. (See the Specification at page 6, lines 1-8; and Examples 1 through 8, pages 7-25, for Claim 9.) The DNA transcription unit can also be administered by contact with a mucosal surface. (See the Specification at page 2, lines 17-21; page 6, lines 4-11; and Example 7, pages 14-15 and 23-24, for Claim 10.) In a preferred embodiment, it is a nasal mucosal surface. (See the Specification at page 6, lines 4-11; and Example 7, pages 14-15 and 23-24, for Claim 13.)

2. Enablement of the Claims

The Specification describes the components of the DNA transcription unit and methods of production of the transcription units (p. 4, lines 19-32). It also describes what constitutes a "desired antigen" (p. 5, lines 6-18). It further describes potential pathogens for which a DNA transcription unit can be used (p. 5, lines 19-33), and routes of inoculation, including mucosal (p. 6, lines 1-11). In the Examples, the Specification describes successful immunization of vertebrates against influenza virus. Influenza exemplifies the success of the method in protecting against disease. A person skilled in the art,

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utilizing the Specification and particularly the description of transcription units, desired antigens, potential pathogens, and routes of inoculation, would be able to make and use other DNA transcription units to immunize vertebrates against diseases caused by other pathogens.

B. Claims 19-20 and 22-23

1. Subject Matter of the Claims

Claim 19 pertains to a method of immunizing a vertebrate against influenza virus, the method comprising administering a DNA transcription unit which comprises DNA encoding an influenza virus antigen linked to a promoter region, thereby eliciting a humoral immune response and/or a cell-mediated immune response against the desired antigen, whereby the vertebrate is protected from disease caused by influenza virus. (See the Specification at page 2, lines 5-16; page 4, lines 5-11; page 6, lines 15-20; and Examples 1 through 8, pages 7-25.)

Claim 22 also pertains to a method of immunizing a vertebrate against influenza virus, the method comprising administering *to a mucosal surface* a DNA transcription unit which comprises DNA encoding an influenza virus antigen linked to a promoter region, thereby eliciting a humoral immune response and/or a cell-mediated immune response against the desired antigen, whereby the vertebrate is protected from disease caused by influenza virus. (See the Specification at page 2, lines 5-21; page 4, lines 5-11; page 6, lines 4-11; and Example 7, pages 14-15 and 23-24 for Claim 22.)

In a preferred embodiment, the virus is the influenza virus and the antigen is hemagglutinin (See the Specification at page 5, lines 15-34; and Examples 1 through 8, pages 7-25, for Claims 20 and 23.)

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2. Enablement of the Claims

In the Examples, the Specification describes successful immunization of vertebrates against influenza virus utilizing DNA transcription units comprising DNA encoding hemagglutinin. The hemagglutinin subtypes used in the Examples are H1 and H7.

One of skill in the art, utilizing the description in the Specification of the DNA transcription units comprising DNA encoding the H1 and H7 hemagglutinin antigens, would be able to make and use other DNA transcription units comprising DNA encoding antigens for other hemagglutinin subtypes. H1 and H7 are representative of the subtypes of influenza against which protection can be achieved.

Rejection of Claims 1, 2 and 4 under 35 U.S.C. 103 over King

The Examiner has maintained the rejection of Claims 1, 2 and 4 over King (Biotechnology News 11(28):5 (1991)), stating that:

> King describes a DNA transcription unit (comprising a CMV early promoter sequence and a TPA sequence) with genes encoding the gp120 of HIV. The transcription unit was injected into the muscle of mice and 'in turn produced cytotoxic T cells against the gp120 protein.'. King appear[s] to meet the limitations set forth in the claims and, absent evidence to the contrary, one would reasonably expect the immune response to be protective. (Advisory Action).

Applicants respectfully disagree with this assessment.

A. Subject Matter of Claims

As described above, Claims 1, 2 and 4 pertain to a method of immunizing a vertebrate against an infectious agent, the method comprising administering a DNA transcription unit which comprises DNA encoding an antigen linked to a promoter region, thereby eliciting a humoral

immune response and/or a cell-mediated immune response against the desired antigen, whereby the vertebrate is protected from disease caused by the infectious agent.

B. Teachings of the Reference Cited

King describes injection into the muscle of a mouse of a construct comprising the gene for gp-120 from the human immunodeficiency virus (HIV), a cytomegalovirus (CMV) early promoter sequence and a tissue plasminogen activator (TPA) sequence. King also describes production of cytotoxic T cells against the gp-120 protein in the mice.

C. Nonobviousness of the Claims

One of ordinary skill in the art would be aware that production of cytotoxic T cells against a protein does not necessarily indicate that a protective effect will be achieved. It is well known in the art that it is necessary to demonstrate that protection accompanies recognition by an antibody response (see Dixon, F.J. and D.W. Fisher, The Biology of Immunologic Disease (HP Publishing Co., New York, 1983), pp. 331-338; a copy of this reference was attached as Exhibit A to Amendment B filed September 1, 1994). For example, cytotoxic T lymphocytes are not a necessary component for protective immunizations against influenza virus in the murine model (see Scherle, P.A. *et al.*, *J. Immunol. 148*:212-217 (1992); Eichelberger, M. *et al.*, *J. Exp. Med. 174*:875 (1991); copies of these references were attached as Exhibits 2 and 3 to the Amendment After Final Rejection filed February 2, 1995). In contrast, in the chicken influenza virus model, cytotoxic T lymphocyte responses have not provided protection (Brown, D. W. *et al.*, *Avian Diseases 36*:515-520 (1992); a copy of this reference was attached as Exhibit 4 to the Amendment After Final Rejection filed February 2, 1995). Thus, it is known in the art that production of

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cytotoxic T cells does not necessarily correlate with protection upon challenge. One of ordinary skill in the art would not have had a reasonable expectation of success in generating a protective immune response.

Furthermore, the construct described by King encodes a protein from a virus that is pathogenic to humans: mice do not develop acquired immune deficiency syndrome (AIDS), the disease caused by the human immunodeficiency virus (HIV). Therefore, King cannot describe protection of a vertebrate from disease. Protection from disease is an important limitation in the rejected claims which is not described in the King reference.

Rejection of Claims 1, 2, 4, 7-14 and 17-24 under 35 U.S.C. 103 over WO 90/11092 in view of Huylebroeck *et al.*

The Examiner has maintained the rejection of Claims 1, 2, 4, 7-14 and 17-24 over WO 90/11092 in view of Huylebroeck *et al.*, stating that:

> [G]iven the concern and focus in the art on effective vaccine against influenza and the fact that the major response to influence infection is directed to the immunodominant hemagglutinin molecule, one would be motivated to include in the DNA transcription unit the gene for hemagglutinin in a method of delivering polynucleotide into a cell. It would have been expected, barring evidence to the contrary, that the immune responses would be protective. (Advisory Action).

Applicants respectfully disagree with this assessment, and submit that one of ordinary skill would not have been motivated to combine the references. Even if the references were combined, the current invention would not have been obvious to one of skill in the art.

A. Teachings of the References Cited

1. WO 90/11092

WO 90/11092 describes methods of delivering RNA or DNA polynucleotides into a vertebrate cell by interstitial delivery, exemplified by mRNA vaccination of mice to produce gp120 protein of the human immunodeficiency virus (HIV). WO 90/11092 indicates that an antibody response was elicited in the mice. WO 90/11092 does not describe any protective immune response.

2. Huylebroeck *et al.*

Huylebroeck *et al.* describe use of DNA in cell culture systems to produce the influenza virus protein hemagglutinin (HA). Huylebroeck *et al.* use recombinant, infectious, replication competent virus vector to express HA. The vector is an SV40 late replacement vector, which is designed to undergo episomal replication in eukaryotic cells. It includes the complete early region of the SV40 genome, as well as the SV40 origin of replication. The late region of the SV40 genome is replaced by a polylinker site that allows cloning of genes in the position for SV40 structural proteins. The expression of the SV40 early region plus the SV40 origin of replication supports episomal replication and amplification of the vector DNA in eukaryotic cells, to enhance levels of protein produced by the vector in the cell culture. The early region of the SV40 genome which supports episomal replication of DNA is the oncogenic region of SV40; the tumor antigens encoded by this region support transformation of cells and tumor induction. Such tumor inducing genes are inappropriate for introduction in vivo.

B. Improper Combination of the References

In order for references to be combined, there must be some teaching or suggestion in the prior art of record

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supporting the combination (ACS Hospital Systems, Inc. v. Montefiore Hospital, 221 USPQ 929, 933 (CAFC 1984)). However, no such teaching or suggestion appears in either WO 90/11092 or in Huylebroeck *et al.* Neither WO 92/11092 nor Huylebroeck *et al.* provide the necessary motivation to combine the references. One of ordinary skill in the art would not have been motivated to look beyond the general teachings of WO 90/11092 concerning delivery of polynucleotides, to the teachings of Huylebroeck *et al.* concerning influenza virus. There is no teaching or suggestion in WO 90/11092 that one of ordinary skill should look to the Huylebroeck *et al.* reference, which teaches influenza virus in particular, as opposed to looking to a reference describing any other possible viruses or pathogens.

Furthermore, one of ordinary skill in the art would not have been motivated to look beyond the teachings of Huylebroeck *et al.* concerning influenza virus, to the teachings of WO 90/11092 concerning the delivery of polynucleotides, because the methods of production of hemagglutinin described in Huylebroeck *et al.* differ in important aspects from the methods of generating desired antigens of the current invention. Huylebroeck *et al.* utilize cell culture systems to generate influenza hemagglutinin proteins used in vaccination. As described above, the vectors employed by Huylebroeck *et al.* are inappropriate for use in humans, as they contain tumor inducing genes. Further, Huylebroeck *et al.* use an infectious agent, replication competent vaccinia virus, to express hemagglutinin in an animal. In contrast, the current invention uses DNA encoding only the particular antigens, such as hemagglutinin, to produce the protein in a vertebrate animal. This DNA does not encode replication-competent virus, and is not capable of replication in the host. Huylebroeck *et al.* do not teach or suggest any other

method of production of influenza hemagglutinin, such as production of influenza hemagglutinin in vivo through DNA inoculation, as in the current invention. One of ordinary skill in the art would not have been motivated by the teachings of Huylebroeck *et al.* to utilize solely the hemagglutinin DNA in order to generate antigen in the organism to be vaccinated.

C. Nonobviousness of the Claims in View of the Combination of References

Obviousness is established only if the teachings of the cited art would suggest the claimed invention to one of ordinary skill in the art with a reasonable degree of certainty of successfully achieving the claimed results. Thus, even if the references were improperly combined, the current invention would not have been rendered obvious, because one of ordinary skill in the art would not have had a reasonable expectation of success in achieving the claimed results.

1. Claims 1, 2, 4, 7-14 and 17-18

As described above, Claims 1, 2, 4 and 7-10 pertain to methods of immunizing a vertebrate against an infectious agent, comprising administering a DNA transcription unit comprising DNA encoding a desired antigen, thereby eliciting a humoral immune response and/or a cell-mediated immune response against the desired antigen, whereby the vertebrate is protected from disease caused by the infectious agent. Claims 11-14 and 17-18 pertain to methods of immunizing a vertebrate against an infectious agent, comprising administering to a mucosal surface a DNA transcription unit comprising DNA encoding a desired antigen, thereby eliciting a humoral immune response and/or a cell-mediated immune response against the desired

antigen, whereby the vertebrate is protected from disease caused by the infectious agent.

Even if WO 90/11092 and Huylebroeck *et al.* were improperly combined, the current invention would not have been obvious to one of ordinary skill in the art. One of ordinary skill in the art would not have had a reasonable expectation that utilization of DNA encoding a particular antigen would result in protection of vertebrate animals against infection and disease. Immune response such as that described in WO 92/11092 is not necessarily indicative of the ability of the vaccine to protect against infection. Furthermore, WO 90/11092 describes vaccination of mice with constructs that encode a protein from the HIV virus, which is pathogenic to humans but not to mice. Therefore, it would not have been obvious to one of ordinary skill in the art that one could protect a vertebrate against any disease. Applicants have, for the first time, demonstrated that inoculation with a DNA transcription unit encoding a desired antigen results in protection from disease caused by the infectious agent.

2. Claims 19-24

As discussed above, Claims 19-20 pertain to methods of immunizing a vertebrate against influenza virus, comprising administering a DNA transcription unit comprising DNA encoding an influenza virus antigen, thereby eliciting a humoral immune response and/or a cell-mediated immune response against the antigen, whereby the vertebrate is protected from disease caused by influenza virus. Claims 22-23 pertain to methods of immunizing a vertebrate against influenza virus, comprising administering to a mucosal surface a DNA transcription unit comprising DNA encoding an influenza virus antigen, thereby eliciting a humoral immune response and/or a cell-mediated immune response against the antigen, whereby the vertebrate is protected from disease

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caused by influenza virus. In the preferred embodiment as claimed in Claims 21 and 24, the hemagglutinin is subtype H1 or H7 (See the Specification at page 5, lines 27-34; and Examples 1 through 8, pages 7-25.)

One of ordinary skill in the art would not have had a reasonable expectation that utilization of DNA encoding hemagglutinin would result in protection of vertebrate animals against influenza. One of ordinary skill in the art would be aware that production of cytotoxic T cells against a protein does not necessarily indicate that a protective effect will be achieved for influenza. As discussed above, cytotoxic T lymphocytes are not a necessary component for protective immunizations against influenza virus in the murine model; in the chicken influenza virus model, cytotoxic T lymphocyte responses have not provided protection. Applicants have, for the first time, shown that inoculation by administering a DNA transcription unit encoding a desired antigen, such as hemagglutinin, results in protection from disease caused by the infectious agent, influenza.

Conclusion

In view of the arguments presented above, Applicants respectfully request that the Examiner reconsider and withdraw all rejections, and submit that the claims are in condition for allowance.

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If the Examiner believes that a telephone conversation will expedite prosecution of this application, the Examiner is requested to call Applicants' Attorney at (617) 861-6240.

Respectfully submitted,

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